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Characterization of Dried Whey Protein Concentrate and Isolate Flavor

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ABSTRACT

The flavor of whey protein concentrates (WPC 80) and whey protein isolates (WPI) was studied using instrumental and sensory techniques. Four WPC 80 and 4 WPI, less than 3 mo old, were collected in duplicate from 6 manufacturers in the United States. Samples were rehydrated and evaluated in duplicate by descriptive sensory analysis. Duplicate samples with internal standards were extracted with diethyl ether. Extracts were then distilled to remove nonvolatile material using high vacuum distillation. Volatile extracts were analyzed using gas chromatography/olfactometry with post peak intensity analysis and aroma extract dilution analysis. Compounds were identified by comparison of retention indices, odor properties, and gas chromatography/mass spectrometry against reference standards. Whey proteins exhibited sweet aromatic, cardboard/wet paper, animal/wet dog, soapy, brothy, cucumber, and cooked/milky flavors, along with the basic taste bitter, and the feeling factor astringency. Key volatile flavor compounds in WPC 80 and WPI were butanoic acid (cheesy), 2-acetyl-1-pyrroline (popcorn), 2-methyl-3-furanthiol (brothy/burnt), 2,5-dimethyl-4-hydroxy-3-(2H)-furanone (maple/spicy), 2-nonenal (fatty/old books), (E,Z)-2,6-nonadienal (cucumber), and (E,Z)-2,4-decadienal (fatty/oxidized). This baseline data on flavor and flavor sources in whey proteins will aid ongoing and future research and will help to identify the most appropriate whey ingredients to use to control or minimize flavor variability in whey enhanced products.
(Key words: whey protein concentrate, whey protein isolate, flavor)

Abbreviation key: AEDA = aroma extract dilution analysis, **$\log_3$FD** = $\log_3$ flavor dilution, **GC/O** = gas chromatography/olfactometry, **RI** = retention index, **WPC** = whey protein concentrates, **WPI** = whey protein isolates.

INTRODUCTION

Dried whey and dried whey products are important ingredients in the food industry. Although liquid whey is not often used as a food ingredient, production exceeded 39 million kg (86 million pounds) in 2004 (USDA, 2005). Liquid whey is further processed into dried whey powder, whey protein concentrates (**WPC**; 35 to 80% protein), and whey protein isolates (**WPI**; >90% protein). Dried whey proteins are commonly used as ingredients due to their exceptional functional characteristics including gelation and viscosity (Morr and Foegeding, 1990). Whey proteins also provide an excellent way to fortify foods with proteins and thus increase their overall nutritional value (Quach et al., 1999).

The flavor of whey is one of the limiting factors in its widespread usage. It has been suggested that off-flavors such as brothy, diacetyl, sourness, and bitterness are the main sensory attributes that limit whey protein usage in bland products (McGugan et al., 1979; Quach et al., 1999). As whey is processed into WPC 80 and WPI, there are many potential sources of flavor formation. Because liquid whey is pooled (sometimes from different types of cheese) before processing into WPC 80 and WPI, there are many sources of flavor variability. Swaisgood (1996) stated that volatile lipid oxidation products were the main sources of off-flavors in both liquid and dried whey, although whey contains only a small amount of lipid. Other studies have confirmed a wide variety of volatile lipid oxidation products in liquid whey and dried whey products, including methyl ketones, aldehydes, and free fatty acids (Hidalgo and Kinsella, 1989; Mills, 1993; Carunchia Whetstine et al., 2003a; Karagul-Yuceer et al., 2003a; Mahajan et al., 2004). Proteolysis is also an important flavor reaction in whey. Proteolytic enzymes, including chymosin, carry over into the whey and may promote the degradation of amino acids, leading to undesirable flavor formation (Holmes et al., 1977; Amundson, 1984). Variability in the type and concentration of free amino acids in whey has also been reported (Mavropoulou and Kosikowski, 1972; Mills, 1993) and may be a source of flavor variability in dried whey products. Proteins may also bind volatile flavor compounds during processing (Stevenson and

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Chen, 1996), thereby contributing to flavor formation in the end product.

Previous studies (Carunchia Whetstine et al., 2003a; Karagul-Yuceer et al., 2003a) showed that metallic and cardboard flavors are present in liquid Cheddar whey. Furthermore, considerable variability in flavor and flavor volatiles existed in liquid Cheddar whey from different starter culture rotations and from different production facilities. Dried whey ingredients have been associated with oxidized, unpleasant flavors that are not pleasing to consumers (Morr and Ha, 1991; Branger et al., 1999). Few studies have specifically addressed the flavor of dried whey or whey proteins (Stevenson and Chen, 1996; Quach et al., 1999; Mahajan et al., 2004). Mahajan et al. (2004) characterized the aroma-active components from 2 samples of sweet whey powder. Sensory properties were not addressed. Other studies have optimized total volatile component analysis (not aroma-active components) (Quach et al., 1999) or evaluated processing methods to minimize volatile component entrapment (Stevenson and Chen, 1996). To our knowledge, the application of quantitative sensory analysis in conjunction with instrumental flavor volatile analysis on multiple whey protein samples has not been conducted. The objectives of this research were to identify and characterize sensory flavor and volatile aroma-active compounds that contribute to flavor in WPC 80 and WPI. Products from different domestic manufacturers as well as different cheese types were evaluated. A previously established defined sensory language for dried dairy ingredients (Drake et al., 2003) was applied by a trained sensory panel in conjunction with instrumental volatile analysis to characterize flavor and flavor variability in this important food ingredient.

MATERIALS AND METHODS

Whey Proteins

Whey protein concentrates (n = 7) and WPI (n = 8) (15 kg, commercially packaged) from different cheese types (Table 1) were received from different US manufacturers within 48 h of production. Duplicate samples were received from each manufacturer in the fall of 2004. Subsamples (500 g) were taken and stored in glass jars flushed with nitrogen, immediately frozen at −80°C, and analyzed within 3 mo of receipt. Whey protein concentrate and WPI samples were reconstituted to 10% solids using deodorized water (prepared by boiling 4 L of distilled water until its volume was decreased by one-third) and blended with a hand-held electric mixer for sensory and instrumental analyses.

Analyses of pH, moisture, fat, protein, and mineral were conducted on all whey proteins in duplicate using standard methods. The pH values were determined by rehydrating the powders and measuring the pH using a pH electrode. Fat content was determined by Mojonnier analysis (Mojonnier Bros. Co., Chicago, IL; Wehr and Frank, 2004). Moisture was determined by the vacuum oven method, and ash content was determined using a muffle furnace. Protein concentration was determined by the Kjeldahl method using a conversion factor of 6.38 to convert total N to protein concentration (Wehr and Frank, 2004). Mineral analysis (calcium, magnesium, potassium, sodium, and phosphate) was conducted using inductively coupled plasma atomic emission spectroscopy.

Table 1. Whey protein concentrate 80 (WPC) and whey protein isolates (WPI) used in the study.[1]

Sample	Manufacturer	Cheese type	Region of US[2]
WPC 80			
WPC 1	1	Primarily Mozzarella	Midwest
WPC 2	1	Primarily Mozzarella	Midwest
WPC 3	2	Mozzarella	Midwest
WPC 4	2	Mozzarella	Midwest
WPC 5	3	Mozzarella	West coast
WPC 6	4	Cheddar	West coast
WPC 7	4	Cheddar	West coast
WPI			
WPI 1	1	Primarily Cheddar	Midwest
WPI 2	1	Primarily Cheddar	Midwest
WPI 3	5	Cheddar	Midwest
WPI 4	5	Cheddar	Midwest
WPI 5	4	Cheddar	West coast
WPI 6	4	Cheddar	West coast
WPI 7	6	Cheddar	West coast
WPI 8	6	Cheddar	West coast

[1]All products were <3 mo old before analysis.

[2]Geographical region of United States in which the product was manufactured.

Descriptive Sensory Analysis

A trained sensory panel (n = 7) evaluated the flavor attributes of the reconstituted whey proteins using a previously published lexicon for dried dairy ingredients (Drake et al., 2003). The definitions and references for the terms used are given in Table 2. Panelists each received 100 h of training on aroma and flavor evaluation of dried dairy ingredients, including both WPI and WPC 80. Flavor and taste intensities were scaled using the 15-point universal intensity scale characterized by the Spectrum descriptive analysis method (Meilgaard et al., 1999; Drake and Civille, 2003). Consistent with Spectrum descriptive analysis training, panelists were presented with reference solutions of sweet, sour, salty, and bitter tastes to learn to consistently use the universal intensity scale (Meilgaard et al., 1999; Drake and Civille, 2003). Following consistent use of the Spectrum

Table 2. References for descriptive sensory analysis of whey proteins.[1]

Term	Definition	Reference	Example/preparation
Overall aroma intensity	The overall orthonasal aroma impact		Evaluated as the lid is removed from the cupped sample
Flavors, tastes, feeling factors (evaluated in the mouth)			
Sweet aromatic	Sweet aroma associated with dairy products		Vanilla cake mix or 20 ppm vanillin in milk
Cooked/milky	Aromatic associated with cooked milk	Cooked milk	Heating skim milk to 85°C for 30 min
Doughy/fatty	Aroma associated with canned biscuit dough	(Z)-4-heptenal	1 ppm (Z)-4-heptenal in water or canned biscuit dough
Cucumber	Aroma associated with freshly sliced cucumber	(E)-2-nonenal	1 ppm (E)-2-nonenal or freshly sliced cucumbers
Brothy	Aromatics associated with broth or boiled potatoes	Methional	1 ppm methional in water or boiled potatoes
Cardboard/wet paper	Aroma associated with cardboard	Cardboard, paper	Cardboard in water
Animal/wet dog	Aroma associated with wet dog hair	Knox gelatin	Dissolve 1 bag of gelatin (28 g) in 2 cups of distilled water
Pasta water	Aroma associated with water after pasta has been boiled in it		Boil pasta in water for 30 min
Soapy	Aroma associated with soap	Lauric acid	1 ppm lauric acid or shaved bar soap
Bitter	Basic taste associated with bitterness	Caffeine	Caffeine, 0.5% in water[2]
Astringency	Drying tongue sensation	Alum	Alum, 1% in water[2]

[1]Adapted from Drake et al., 2003; Karagul-Yuceer et al., 2003a.
[2]Meilgaard et al., 1999.

scale with basic tastes, panelists learned to identify and scale flavor descriptors using the same intensity scale through presentation and discussion of flavor definitions, references (Table 2), and a wide array of rehydrated dried dairy ingredients. Samples were reconstituted 24 h before evaluation at 10% solids using deodorized water and stored at 5°C. Samples were tempered to 12°C and evaluated in duplicate by each panelist in a randomized balanced block design on separate occasions in 125-mL lidded plastic cups identified with 3-digit random codes.

Chemicals

Diethyl ether (anhydrous, 99.8%), sodium chloride (99%), sodium sulfate (99%), and 2-methyl-3-heptanone (internal standard for neutral/basic fraction) were obtained from Aldrich Chemical Company (St. Louis, MO). Compounds in Tables 7 and 8 were provided by Aldrich (St. Louis, MO) with the following exceptions: 1-octen-3-one was obtained from Lancaster (Windham, NH), 4-methoxyphenol was obtained from TCI America (Portland, OR) and 2-acetyl-1-pyrroline was not commercially available. Sodium bicarbonate (99.7%) and hydrochloric acid (36.5%) were obtained from Fisher Scientific (Pittsburgh, PA).

Volatile Extract Preparation

Direct solvent extraction. Whey protein concentrate and WPI volatile extracts were prepared using the methods of Milo and Reineccius (1997). Whey proteins (20 g) were reconstituted to 10% solids in duplicate and divided into 4 Teflon bottles (capacity of 250 mL) with Tefzel closures per duplicate. Two hundred milliliters of diethyl ether (50 mL/bottle) with 10 μL of internal standard (10 μL of 2-methyl-3-heptanone and 50 μL of 2-methyl pentanoic acid in 5 mL of methanol) and 60 g of NaCl were combined and equally distributed among each bottle. The mixtures were shaken for 30 min on a Roto mix (Type 50800; Thermolyne, Dubuque, IA) at high speed. The bottles were then centrifuged at 735 × *g* for 15 min to separate the nonpolar solvent phase from the mixture. This solvent phase was subsequently removed via pipette and saved in a glass jar. The procedure was repeated twice with the addition of 50 mL of diethyl ether to each bottle. The third addition was centrifuged at 325 × *g*. The solvent phases were combined and dried over anhydrous sulfate and concentrated to 120 mL using a Vigreux column (Fisher Scientific, Allentown, PA).

High-vacuum distillation. Volatile compounds from WPC 80 and WPI extracts were separated using a high vacuum distillation technique detailed by Karagul-Yuceer et al. (2001). The distillation process began by placing the extract into a 1-L round-bottomed flask and immersing it into a Dewar vessel containing liquid nitrogen until shell frozen. The frozen flask was then immediately connected to a distillation unit equipped with a rough pump/diffusion pump as the vacuum source (about 10^{-4} Torr), a receiving tube, and a waste tube. The receiving tube and waste tube were held in

separate Dewar vessels containing liquid nitrogen until distillation was completed (4 h). For the first 2 h, the sample flask was held at room temperature. During the second 2 h, the sample was kept at constant temperature in a water bath (50°C). After distillation, the distillate was concentrated to 20 mL under a stream of nitrogen gas. The concentrated distillate was then washed twice with 3 mL of sodium bicarbonate (0.5 *M*) and vigorously shaken. It was then washed 3 times with 2 mL of saturated sodium chloride solution. The upper layer (ether) containing the neutral/basic fraction was collected in a glass tube using a pipette. The distilled extracts were then dried over anhydrous sodium sulfate and concentrated to 0.5 mL under a stream of nitrogen gas. Acidic volatiles were recovered by acidifying the bottom layer (aqueous phase) with about 5 mL of 6.2 *M* hydrochloric acid to pH 2 to 2.5 and extracting the sample 3 times with 5 mL of diethyl ether. The extracted acidic volatiles were then dried over anhydrous sodium sulfate before concentration to 0.5 mL under nitrogen.

Gas Chromatography/Olfactometry

Two methods of sniffing were used for the evaluation of aroma-active volatile compounds in the extracts. Post peak intensity was conducted on all fractions of all samples. Representative samples were then analyzed using aroma extract dilution analysis (**AEDA**; Grosch, 1993; Van Ruth, 2001).

Post peak intensity. An HP5890 series II gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame-ionization detector, a sniffing port, and a splitless injector was used for gas chromatography/olfactometry (**GC/O**). Both the neutral/basic and acidic fractions were analyzed from each duplicate extraction. Two microliters was injected onto a polar capillary column (DB-WAX, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness; J&W Scientific, Folsom, CA) and a nonpolar column (DB-5MS, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness; J&W Scientific). Column effluent was split 1:1 between the flame-ionization detector and sniffing port using deactivated fused silica capillaries (1 m length × 0.25 mm i.d.). The oven temperature was programmed from 40 to 200°C at a rate of 10°C/min with an initial hold for 3 min and a final hold of 20 min. The flame-ionization detector and sniffing port were maintained at a temperature of 250°C. The sniffing port was supplied with humidified air at 30 mL/min. Post peak intensity was used to characterize the odorants in the extracts (Van Ruth, 2001). Two experienced panelists (each with more than 150 h of experience) sniffed the neutral/basic and acidic fractions of the WPC80 and WPI extracts twice on the 2 columns. Sniffers described the odor and scored the intensity of odorants in the extracts using a 10-point numerical intensity scale (Van Ruth, 2001).

AEDA. The same conditions and system were used for AEDA analysis as in post peak intensity. Representative samples were selected for AEDA based on aroma-active volatile differences identified during post peak intensity and by flavor differences identified by descriptive sensory analysis. Four representative WPC 80 (samples 2, 3, 5, and 6) and 3 representative WPI (samples 2, 4, and 7) were analyzed (Table 1). Each neutral/basic extract was injected onto a DB-5MS capillary column (30 m length × 0.25 mm i.d. × 0.25 μm d_f; J&W Scientific, Folsom, CA) and each acidic fraction was injected on to a DB-WAX capillary column (30 m length × 0.25 mm i.d. × 0.25 μm film thickness; J&W Scientific) under the above conditions. The extracts were diluted stepwise with diethyl ether at a ratio of 1/3 (vol/vol). Two experienced sniffers with more than 150 h training on GC/O evaluated each dilution. The dilution procedure was repeated until sniffers detected no odorants. The highest dilution was reported as the flavor dilution (**log FD_3**) factor (Grosch, 1993).

Gas Chromatography/Mass Spectrometry

Gas chromatography/mass spectrometry analysis of the extracts used a HP5890 Series II GC/HP 5972 mass selective detector (Hewlett-Packard). Separations were performed on a fused silica capillary column (DB-5MS, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific). Helium gas was used as a carrier at a constant flow of 1 mL/min. Oven temperature was programmed from 40 to 200°C at a rate of 5°C/min with initial and final hold times of 5 and 45 min, respectively. Mass selective detector conditions were as follows: capillary direct interface temperature, 280°C; ionization energy, 70 eV; mass range, 33 to 330 amu; scan rate, 5 scans/s. Each extract (2 μL) was injected in the splitless mode. Duplicate analyses were performed on each sample.

Identification of odorants. For positive identifications, retention indices (**RI**), mass spectra, and odor properties of unknowns were compared with those of authentic standard compounds analyzed under identical conditions. Tentative identifications were based on comparing mass spectra of unknown compounds with authentic standards or on matching the RI values and odor properties of unknowns against those of authentic standards. For the calculation of retention indices, an n-alkane series was used (Van den Dool and Kratz, 1963).

Quantification of odorants. Selected compounds were quantified. The area ratio (area of internal standard/area of compound) was multiplied by the concen-

Table 3. Proximate analysis of whey protein concentrate (WPC 80) from different manufacturers.[1]

	Manufacturer 1		Manufacturer 2		Manufacturer 3	Manufacturer 4	
Attribute	WPC 1	WPC 2	WPC 3	WPC 4	WPC 5	WPC 6	WPC 7
pH	6.73[a]	5.83[e]	6.25[b]	6.23[b]	5.89[c]	6.23[b]	6.60[a]
Ash (% dry weight basis)	3.13[a]	3.17[a]	2.87[b]	2.59[cd]	2.48[d]	2.79[b]	2.72[bc]
Moisture (%)	4.09[b]	3.77[d]	3.97[c]	3.25[f]	3.52[e]	4.51[a]	4.02[bc]
Protein (% dry weight basis)	76.52[cd]	76.75[cd]	74.78[e]	76.06[d]	77.44[bc]	78.2[b]	80.00[a]
Fat (% dry weight basis)	6.29[a]	6.27[a]	4.53[d]	4.87[c]	5.39[b]	5.41[b]	5.55[b]
Calcium (mg/100 g)	846.5[a]	829.0[a]	509.5[cd]	489.0[d]	378.5[e]	529.5[bc]	548.0[b]
Magnesium (mg/100 g)	31.35[d]	30.70[d]	59.25[ab]	64.25[a]	58.00[b]	53.20[c]	57.80[b]
Potassium (mg/100 g)	388.0[c]	384.0[c]	510.0[b]	506.5[b]	562.5[a]	519.0[b]	519.0[b]
Sodium (mg/100 g)	150.0[bc]	144.5[cd]	151.0[bc]	138.0[d]	164.5[a]	157.0[ab]	151.0[bc]
Phosphate (mg/100 g)	364.5[a]	356.5[ab]	349.5[bc]	343.0[ab]	318.0[e]	339.5[cd]	330.5[de]

[a–f]Means in a row followed by different letters are different ($P < 0.05$).

[1]WPC samples from different manufacturing sites (as listed in Table 1).

tration of the internal standard to determine the relative abundance of the compound. The neutral/basic compounds were quantified from a DB-5MS column and acids were quantified using a DB-WAX column.

Statistical Analyses

Proximate analysis, sensory, and instrumental quantitation results were analyzed using the SAS statistical software (version 8.2, SAS Institute, Cary, NC). Proximate analysis and instrumental data were treated as a completely randomized design with repeated measures. Sensory data were treated as a randomized balanced block with repeated measures. Analysis of variance with means separation (least squares means) was conducted to identify differences among products.

RESULTS AND DISCUSSION

WPC 80 and WPI Composition

There were compositional differences among the different WPC 80 and WPI ($P < 0.05$; Tables 3 and 4). The pH of these samples ranged from 5.8 to 6.9. There were no consistent differences between products made from Cheddar and Mozzarella whey. Although there were statistically significant differences in composition, products made from the same manufacturing site had similar proximate compositions and all products were within expected ranges for their proximate composition (Jelen, 2000). Due to the high degree of reproducibility within these measurements, statistical differences were observed among the WPC80 and the WPI, but these differences are likely not important from a practical standpoint as they pertain to the objectives of this study—the flavor of fresh dried whey proteins.

The main compositional differences identified were in the mineral content. This is expected because each facility has different ways of concentrating and processing the liquid whey. Different filtration and processing procedures may selectively filter out different minerals and salts. Additionally, WPC samples 1 through 5 were produced mainly from Mozzarella whey, whereas WPC samples 6 and 7 were produced from Cheddar whey; this could also contribute to some of the

Table 4. Proximate analysis of whey protein isolate (WPI) samples from different manufacturers.[1]

	Manufacturer 1	Manufacturer 5		Manufacturer 4		Manufacturer 6	
Attribute	WPI 2	WPI 3	WPI 4	WPI 5	WPI 6	WPI 7	WPI 8
pH	6.92[a]	5.92[c]	5.97[c]	6.05[bc]	6.14[b]	6.22[b]	5.91[c]
Ash (%, dry weight basis)	2.02[d]	2.40[b]	2.17[c]	1.96[d]	2.19[c]	2.43[b]	2.54[a]
Moisture (%)	4.15[d]	6.04[b]	6.03[b]	6.65[a]	5.32[c]	3.37[f]	3.68[e]
Protein (%, dry weight basis)	93.94[a]	88.22[d]	87.90[ed]	87.69[e]	85.85[f]	89.88[b]	89.28[c]
Fat (%, dry weight basis)	0.66[b]	0.14[de]	0.25[d]	0.19[de]	0.06[e]	0.50[c]	0.82[a]
Calcium (mg/100 g)	56.6[e]	477.0[c]	459.0[d]	486.5[c]	453.5[d]	525.0[b]	554.5[a]
Magnesium (mg/100 g)	2.5[e]	74.8[c]	80.5[cd]	65.7[d]	67.4[d]	122.5[b]	129.0[a]
Potassium (mg/100 g)	41.4[e]	433.0[b]	415.5[c]	385.0[d]	525.0[a]	422.0[c]	451.5[b]
Sodium (mg/100 g)	789.0[a]	147.5[c]	132.5[cd]	108.5[d]	159.5[c]	231.5[b]	219.0[b]
Phosphate (mg/100 g)	63.4[f]	224.5[b]	212.0[c]	154.5[e]	197.0[d]	219.0[bc]	288.5[a]

[a–f]Means in a row followed by different letters are different ($P < 0.05$).

[1]WPI samples from different manufacturing sites (as listed in Table 1); data were not available for WPI 1.

Table 5. Descriptive sensory analysis of whey protein concentrate (WPC 80) from different manufacturers.[1,2]

	Manufacturer 1		Manufacturer 2		Manufacturer 3	Manufacturer 4	
Attribute	WPC 1	WPC 2	WPC 3	WPC 4	WPC 5	WPC 6	WPC 7
Aroma intensity	3.00[a]	2.00[b]	3.00[a]	1.75[b]	3.50[a]	3.50[a]	3.25[a]
Sweet aromatic	ND	ND	2.90[a]	2.00[b]	1.50[c]	ND	ND
Cooked/milky	ND	ND	ND	ND	ND	2.00[a]	1.52[a]
Cardboard/wet paper	3.25[a]	2.00[b]	ND	1.50[b]	ND	ND	3.25[a]
Brothy	ND	ND	ND	ND	ND	1.50[a]	ND
Pasta water	ND	1.50[c]	1.50[c]	2.50[bc]	3.25[ab]	4.00[a]	2.50[bc]
Doughy/fatty	ND	ND	1.50[a]	ND	ND	ND	ND
Astringency	3.40[a]	3.00[ab]	2.75[ab]	2.50[b]	3.00[ab]	2.50[b]	2.25[b]

[a,b,c]Means in a row followed by different letters are different ($P < 0.05$).

[1]WPC samples from different manufacturing sites (as listed in Table 1).

[2]Intensities are scored on a 15-point universal scale where 0 = none and 15 = very high (Meilgaard et al., 1999). ND = Not detected consistently by all panelists; mean intensity score <0.50.

observed variability in mineral composition. Previous studies have documented the remarkable variability in the composition of WPC 80 and WPI (Holt et al., 1999). These differences in composition, particularly ash, minerals, moisture, and fat, may play a crucial role in the flavor stability of these products with storage.

Sensory Analysis

Sensory profiles of rehydrated whey proteins are presented in Tables 5 and 6. There were no consistent differences between products made from Cheddar and Mozzarella whey ($P < 0.05$). For both WPC 80 and WPI, significant differences ($P < 0.05$) in the sensory profiles were observed. Two general groups of aromatic flavors were found in the whey proteins—dairy and nondairy flavors. Drake et al. (2003) also documented these groups of flavors in skim and whole milk powder as well as dried whey ingredients and described the flavors as those flavors generally associated with fresh fluid milk or whey (dairy flavors: sweet aromatic, cooked/milky) and those flavors not generally associated with fresh fluid milk or whey (nondairy flavors: cardboard, animal/wet dog, cucumber, etc.).

There were sensory differences among the WPC 80 made from different manufacturing sites. Samples 1, 2, 4, and 7 had high intensities of cardboard/wet paper. Cardboard/wet paper has traditionally been a descriptor for oxidized milk fat (Morr and Ha, 1991), and this flavor has been documented in off-flavored skim and whole milk powder (Drake et al., 2003; Caudle et al., 2005). Samples 4 through 7 also had high intensities of pasta water flavor. Sweet aromatic and cooked/milky were 2 dairy flavors observed in the WPC80. All of the WPC 80 samples were astringent.

The WPI had similar flavor profiles to the WPC 80, but the attributes soapy, animal/wet dog, cucumber, and bitter (all nondairy flavors) were observed only in WPI (not in WPC 80). Delicate dairy flavors, sweet aromatic and cooked/milky, were not observed in the WPI. Isolate samples 1 and 2 had the highest intensities of soapy flavors, but displayed the lowest overall aroma intensities. Samples 3 through 8 were characterized by cardboard/wet paper flavor. Sample 6 was the only WPI

Table 6. Descriptive sensory analysis of whey protein isolate (WPI) samples from different manufacturers.[1,2]

	Manufacturer 1		Manufacturer 5		Manufacturer 4		Manufacturer 6	
Attribute	WPI 1[1]	WPI 2[1]	WPI 3[5]	WPI 4[5]	WPI 5[4]	WPI 6[4]	WPI 7[6]	WPI 8[6]
Aroma intensity	1.75[cd]	1.50[d]	2.50[bc]	3.40[ab]	4.15[a]	3.65[a]	4.00[a]	4.25[a]
Cardboard/wet paper	ND	ND	2.00[ab]	2.25[a]	1.50[b]	2.25[a]	2.00[ab]	2.15[ab]
Animal/wet dog	ND	ND	ND	1.50[b]	2.50[a]	ND	1.50[ab]	1.50[ab]
Soapy	3.00[a]	2.50[a]	ND	ND	ND	1.50[b]	1.50[b]	ND
Brothy	ND	ND	ND	ND	ND	ND	2.00[a]	1.00[b]
Pasta water	ND	ND	ND	ND	ND	2.00	ND	ND
Cucumber	ND	ND	ND	1.50	ND	ND	ND	ND
Bitter taste	1.25[a]	1.00[a]	ND	ND	0.58[a]	0.50[a]	ND	ND
Astringency	2.00[c]	1.50[c]	3.00[a]	2.75[ab]	3.40[a]	1.75[bc]	2.50[abc]	2.50[abc]

[a–d]Means in a row followed by different letters are different ($P < 0.05$).

[2]Intensities are scored on a 15-point universal scale where 0 = none and 15 = very high (Meilgaard et al., 1999). ND = Not detected consistently by all panelists; mean intensity score <0.50.

with pasta water flavor, and sample 4 was the only one with cucumber flavor. These flavors may be formed via lipid oxidation. There were small but significant differences in the fat content of the different WPI, however, there was no clear trend that higher fat content (within the range observed) was associated with increased lipid oxidation flavors such as cardboard/wet paper, cucumber, doughy/fatty, or pasta water. A lack of correlation between fat content and lipid oxidation flavors was also observed with WPC 80. As mentioned previously, it is possible that the observed differences in fat content among the WPI and WPC 80 samples might contribute to differences in flavor stability with increased storage time, even though they do not appear to impact flavor in the fresh product. Additional research would be necessary to confirm this hypothesis.

Though not observed in WPC 80, several of the WPI had animal/wet dog flavor. Animal/wet dog flavor is likely caused by protein degradation and has been previously identified as a characteristic flavor in caseins and caseinates (Karagul-Yuceer et al., 2003b; Drake et al., 2003). Whey protein isolates are high in protein, like caseins and caseinates, further associating this flavor with high protein products. Bitterness and astringency have been associated with proteolysis (Harwalker et al., 1993; Lee et al., 1996a; N'Kouka et al., 2004). The WPI samples 1, 2, 5, and 6 had distinct bitter taste intensities. Bitterness was only observed in the WPI, not in the (lower protein) WPC 80, suggesting that as the protein content increases, so does the potential for bitterness. Consistently bland whey proteins should be the industry ideal. Recent research with low heat skim milk powder has demonstrated that low intensities of nondairy flavors (off-flavors) in milk powders carry through into ingredient applications, and negatively impact consumer acceptance (Caudle et al., 2005). Nondairy or off-flavor intensities in whey proteins are comparable to those observed in milk powders (Drake et al., 2003) and it is reasonable to assume that similar off-flavor carry-through potential exists with whey protein ingredient applications.

GC/O and Odorant Quantification

Post peak intensity aroma analysis of all samples was conducted (data not shown). The WPC 80 samples 1 and 2 were very similar, as were 3 and 4, and 6 and 7. Whey protein isolate samples 1 and 2 were similar to each other, 3 and 4 were similar, and samples 5 through 8 were similar to each other. These results indicate, not surprisingly, that whey proteins from the same facilities have similar (but not necessarily identical) sensory and instrumental flavor profiles. From these preliminary results and descriptive sensory data, 4 representative WPC 80 (samples 2, 3, 5, and 6) and 3 representative WPI (samples 2, 4, and 7) were selected to further pinpoint aroma-active compounds by using the semiquantitative sniffing technique, AEDA. Forty-one potent ($\log_3$ FD <1) aroma-active compounds in WPC and 28 potent ($\log_3$ FD <1) aroma-active compounds in WPI were detected (Tables 7 and 8). In both WPC 80 and WPI there were aldehydes, ketones, and free fatty acids. The aldehydes hexanal, octanal, nonanal, and decanal have been previously identified in both liquid and dried whey ingredients (Lee et al., 1996b; Stevenson and Chen, 1996; Carunchia Whetstine et al., 2003a; Karagul-Yuceer et al., 2003a). These compounds are formed during lipid oxidation as the oxidation of lipids produces a wide range of compounds including aldehydes, ketones, and alcohols (Frankel et al., 1981). Low molecular weight aldehydes have low aroma thresholds (Kinsella et al., 1967; Rychlik et al., 1998) and therefore contribute to flavor. Because there is less fat in WPI than in WPC 80 (approximately 0.5% vs. 5%, respectively), there were fewer aroma-active lipid oxidation products identified in WPI compared with WPC 80.

1-Octen-3-one, dienals, and (Z)-4-heptenal have all been implicated as sources of off-flavors in dairy products that have undergone oxidation (Morr and Ha, 1991). Unsaturated aldehydes have been previously identified in sweet whey powder (Mahajan et al., 2004) and are formed from the autooxidation of unsaturated fatty acids. Hexanal (Karagul-Yuceer et al., 2002), 1-octen-3-one (Day et al., 1963), (Z)- and (E)-2-nonenal (Grosch et al., 1994), (E,Z)-2,6-nonadienal, and (E,E)-2,4-decadienal (Karagul-Yuceer et al., 2001, 2002) likely contribute to cardboard or metallic flavors in liquid whey and dried whey products (Karagul-Yuceer et al., 2003a; Drake et al., 2003). There were no large or consistent differences in the $\log_3$ flavor dilution values for hexanal (grassy), Z-4-heptanal (fatty/fishy), or octanal (citrus/green) among the WPC 80 samples. However, WPC 80 sample 1 contained more fat than WPC 80 samples 3, 5, and 6 (Table 3); higher flavor dilution values for decanal (fatty) and the unsaturated aldehyde (E,E)-2,4-decadienal (oxidized) were also documented in this sample (Table 7). The relative concentration of the short-chain aldehydes was higher in the WPC 80 than in the WPI (Tables 9 and 10), consistent with the higher fat content of the WPC 80. These short-chain aldehydes have low aroma thresholds. However, low molecular weight compounds may be entrapped by the protein in WPC 80 and WPI (Stevenson and Chen, 1996), which would increase their threshold values. Therefore, the threshold values for these compounds in spray-dried WPC 80 and WPI may be much higher than previously reported threshold values in water or buffer.

Table 7. Aroma extract dilution analysis of whey protein concentrate (WPC) samples.

No.	Compound	Fraction	Odor[1]	Log_3 Flavor dilution[2]				Retention index[3]		Methods of identification[4]
				WPC 2	WPC 3	WPC 5	WPC 6	DB-5 column	DB-WAX column	
1	Acetic acid	Ac	Vinegar	2	<1	1	<1		1424	RI, odor, MS
2	2,3 Butanedione	N/B	Buttery	<1	<1	1	ND	680	955	RI, odor, MS
3	Dimethyl disulfide	N/B	Garlic/rubbery	ND	<1	1	<1	777	1071	RI, odor
4	Hexanal	N/B	Green grass	<1	<1	2	<1	803	1051	RI, odor, MS
5	Unknown	N/B	Ammonia	<1	ND	2	ND	824	1140	odor
6	Butanoic acid	Ac	Cheesy/rancid	4	2	3	4	840	1650	RI, odor, MS
7	Unknown	N/B	Citrus	ND	1	<1	<1	856	1072	RI, odor
8	Z-4-Heptenal	N/B	Fatty/fishy	<1	<1	1	1	905	1220	RI, odor, MS
9	Methional	N/B	Potato	1	<1	<1	1	915	1433	RI, odor
10	2-Acetyl-1-pyrroline[5]	N/B	Popcorn	4	<1	3	3	926	1317	RI, odor
11	Dimethyl trisulfide	N/B	Cabbage	<1	1	2	<1	960	1362	RI, odor
12	1-Octen-3-one	N/B	Mushroom	1	ND	1	3	980	1285	RI, odor
13	Octanal	N/B	Citrus/green	1	<1	1	<1	1005	1275	RI, odor
14	Hexanoic acid	Ac	Sweaty	ND	<1	<1	2	1045	1850	RI, odor, MS
15	(E)-2-Octenal	N/B	Citrus/fatty	<1	ND	1	ND	1070	1345	RI, odor, MS
16	2,5-Dimethyl-4-hydroxy-3-(2H) furanone (Furaneol)	Ac	Burnt sugar	1	2	2	ND	1072	2047	RI, odor
17	2-Methyl-3-furanthiol[6]	N/B	Brothy/burnt	<1	2	1	3	1082	1403	RI, odor
18	2-Methoxy phenol (guiacol)	N/B	Smoky	<1	2	3	1	1095	1464	RI, odor
19	Nonanal	N/B	Fatty/citrus	<1	<1	<1	<1	1098	1385	RI, odor, MS
20	3-Hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon)	Ac	Maple/spicy	3	2	3	4	1120	2210	RI, odor
21	2-Phenethanol[6]	N/B	Rosey	1	<1	1	3	1150	1873	RI, odor
22	(E,Z)-2,6-Nondienal	N/B	Cucumber	5	2	1	3	1160	1555	RI, odor
23	(E)-2-Nonenal	N/B	Cucumber/old books	4	<1	1	2	1168	1582	RI, odor, MS
24	2-Isobutyl-3-methoxypyrazine	N/B	Earthy/bell pepper	1	<1	ND	<1	1179	1520	RI, odor
25	Decanal	N/B	Fatty	3	1	ND	1	1223	1483	RI, odor, MS
26	Phenylethyl acetate[6]	N/B	Rosey	<1	<1	<1	1	1245	1820	RI, odor
27	Unknown	N/B	Cilantro	ND	2	1	ND	1261		Odor
28	Unknown	N/B	Oatmeal	2	<1	ND	<1	1280		Odor
29	o-Aminoacetophenone[6]	N/B	Grape	2	<1	<1	ND	1320	2223	RI, odor
30	(E,E)-2,4-Decadienal	N/B	Fatty/oxidized	4	<1	1	ND	1330	1710	RI, odor, MS
31	γ-Nonalactone[6]	N/B	Coconut	<1	1	2	ND	1350	2011	RI, odor
32	Unknown	N/B	Paper/metallic	3	<1	1	ND	1390		Odor
33	4-Methyl octanoic acid[6]	Ac	Waxy/soapy	ND	<1	ND	3	1400	2173	RI, odor
34	3-Methoxy-4-hydroxybenzaldehyde (vanillin)[6]	Ac	Vanilla	<1	1	<1	ND	1435	1892	RI, odor
35	Unknown	N/B	Peach	ND	ND	1	ND	1443		Odor
36	Unknown	Ac	Waxy	ND	<1	1	ND	1454	2216	RI, odor
37	γ-Decalactone	N/B	Peach	1	1	<1	<1	1490	2103	RI, odor
38	δ-Decalactone	N/B	Coconut	<1	<1		1	1510	1990	RI, odor, MS
39	Unknown	N/B	Waxy/soapy	<1	1	<1	ND	1630	1972	RI, odor
40	δ-Dodecalactone	N/B	Peach	1	<1	1	ND	1675		RI, odor, MS
41	Unknown	N/B	Plastic/soap	ND	<1	<1	1	1789		Odor

[1]Odor description at the gas chromatograph (GC) sniffing port.

[2]Flavor dilution factors were determined on a DB-5MS column for neutral/basic (N/B) compounds, and on a DB-WAX column for acidic (Ac) compounds.

[3]Retention indices were calculated from gas chromatography/olfactory data.

[4]Compounds were identified by comparison with the authentic standards on the following criteria: retention index (RI) on DB-WAX and DB-5MS columns, odor property at the GC sniffing port, and mass spectra in the electron impact mode. Positive identifications indicate that mass spectral data was compared with authentic standards.

[5]Compound identified by comparing RI and aroma with literature (Avsar et al., 2004).

[6]Compound not previously identified as an aroma-active constituent of whey (Mills, 1993; Stevenson and Chen, 1996; Carunchia Whetstine et al., 2003a; Karagul-Yuceer et al., 2003a; Mahajan et al., 2004).

This hypothesis would explain why there does not appear to be a direct correlation between the concentration of aldehydes and the sensory perception of off-flavor intensity in fresh products (Tables 4, 5, 9, and 10). Carunchia Whetstine and Drake (2005) also observed inconsistent correlations between total aldehyde concentrations and off-flavors in skim milk powder less than 6 mo old.

Table 8. Aroma extract dilution analysis of representative whey protein isolate (WPI) samples.

No.	Compound	Fraction	Odor[1]	Log_3 Flavor dilution[2]			Retention index[3]		Methods of identification[4]
				WPI 2	WPI 4	WPCI 7	DB-WAX column	DB-5MS column	
1	Acetic acid	Ac	Vinegar	2	ND	2		1424	RI, odor, MS
2	Dimethyl disulfide	N/B	Garlic/rubbery	<1	1	1	777	1071	RI, odor
3	Hexanal	N/B	Green grass	<1	<1	<1	803	1051	RI, odor, MS
4	Butanoic acid	Ac	Cheesy/rancid	4	3	3	840	1650	RI, odor
5	Methional	N/B	Potato	2	5	<1	915	1433	RI, odor
6	2-Acetyl-1-pyrroline[5]	N/B	Popcorn	3	4	1	926	1317	RI, odor
7	Dimethyl trisulfide	N/B	Cabbage	<1	6	3	960	1362	RI, odor
8	1-Octen-3-one	N/B	Mushroom	1	<1	<1	980	1285	RI, odor
9	Octanal	N/B	Citrus/green	1	1	<1	1005	1275	RI, odor, MS
10	Hexanoic acid	Ac	Sweaty	1	3	1	1045	1819	RI, odor, MS
11	2,5-Dimethyl-4-hydroxy-3-(2H) furanone (Furaneol)	Ac	Burnt sugar	<1	1	<1	1072	2047	RI, odor
12	2-Methyl-furanethiol[6]	N/B	Brothy/burnt	ND	4	1	1088	1403	RI, odor
13	Nonanal	N/B	Fatty/citrus	<1	<1	<1	1098	1385	RI, odor, MS
14	Unknown	N/B	Oxidized/phenolic	1	2	<1	1106		Odor
15	3-Hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon)	Ac	Maple/spicy	3	ND	2	1120	2210	RI, odor
16	2-Phenethanol[6]	N/B	Rosey	<1	1	1	1150	1873	RI, odor
17	(E,Z)-2,6-Nondienal	N/B	Cucumber	4	4	<1	1160	1555	RI, odor
18	(E)-2-Nonenal	N/B	Cucumber/old books	3	4	<1	1168	1582	RI, odor, MS
19	Decanal	N/B	Fatty	3	1	2	1223	1483	RI, odor, MS
20	Unknown	N/B	Oatmeal	2	<1	<1	1280		Odor
21	*o*-Aminoacetophenone[6]	N/B	Grape	2	<1	1	1320	2223	RI, odor
22	(E,E)-2,4-Decadienal	N/B	Fatty/oxidized	4	<1	<1	1330	1710	RI, odor
23	Unknown	N/B	Paper/metallic	3	ND	<1	1379		Odor
24	4-Methyl octanoic acid[6]	Ac	Waxy/soapy	ND	1	ND	1400	2173	RI, odor
25	δ-Decalactone	N/B	Coconut	<1	1	<1	1510	1990	RI, odor, MS
26	Unknown	N/B	Fruity	1	ND	ND	1518		Odor
27	Unknown	N/B	Fecal/spicy	<1	1	ND	1547		Odor
28	δ-Dodecalactone	N/B	Peach	1	ND	<1	1675		RI, odor

[1]Odor description at the gas chromatograph (GC) sniffing port.

[2]Flavor dilution factors were determined on a DB-5MS column for neutral/basic (N/B) compounds, and on a DB-WAX column for acidic (Ac) compounds.

[3]Retention indices were calculated from gas chromatography/olfactory data.

[4]Compounds were identified by comparison with the authentic standards on the following criteria: retention index (RI) on DB-WAX and DB-5MS columns, odor property at the GC-sniffing port, and mass spectra in the electron impact mode. Positive identifications indicate that mass spectral data was compared with authentic standards.

[5]Compound identified by comparing RI and aroma with literature (Avsar et al., 2004).

[6]Compound not previously identified as an aroma-active constituent of whey (Mills, 1993; Stevenson and Chen, 1996; Carunchia Whetstine et al., 2003a; Karagul-Yuceer et al., 2003a; Mahajan et al., 2004).

There were several fatty acids identified in both WPC 80 and WPI. Previous studies have identified many different short-chain fatty acids in whey, ranging in chain length from C2 to C18 (Lee et al., 1996b; Tomaino et al., 2001; Karagul-Yuceer et al., 2003a). Acetic acid, butanoic acid, hexanoic acid, and 4-methyl octanoic acid were the only acids identified by GC/O (i.e., displaying aroma activity). Butanoic and hexanoic were found in similar concentration ranges (1 to 10 ppm) as those previously reported in liquid whey (Karagul-Yuceer et al., 2003a). The free fatty acids C4-C8 have lower sensory threshold values than the fatty acids larger than C10 (Tables 9 and 10), which is why they play a larger role in flavor (Karagul-Yuceer et al., 2003a). Octanoic, nonanoic, and decanoic acids were detected by gas chromatography/mass spectrometry only (Tables 9 and 10).

The branched-chain fatty acid 4-methyl octanoic acid has not been previously identified in whey. This compound is not found in high concentrations in cows' milk, but is found in high concentrations in goat and sheep milk (3, 223, and 80 ppm, respectively; Ha and Lindsay, 1991). Carunchia Whetstine et al. (2003b) found that this acid, in conjunction with 4-ethyl octanoic acid, was the source of waxy/animal flavor in goat cheese. The odor threshold of this compound is around 20 ppb at pH 2.0 and the retronasal threshold is 600 ppb in water (Brennand et al., 1989). In all WPC 80 and most of the WPI samples, 4-methyl octanoic acid was present below sensory threshold. In WPI sample 1, 4-methyl octanoic acid was above threshold and might have contributed to the soapy/waxy flavor in this product. However, this acid was present in other samples that did not have

Table 9. Relative abundance of selected compounds in whey protein concentrate (WPC) samples (ND = not detected).

Compound	RI on DB-5MS[1]	Concentration (ppb)							Reported threshold (ppb)
		WPC 1	WPC 2	WPC 3	WPC 4	WPC 5	WPC 6	WPC 7	
Hexanal	803	1300^{a}	390^{bc}	550^{b}	110^{c}	300^{bc}	140^{c}	540^{b}	10.4[3]
Octanal	1005	140^{a}	40^{b}	60^{b}	20^{b}	60^{b}	30^{b}	60^{b}	8[3]
(E)-2-Octenal	1070	70^{a}	20^{a}	ND	ND	20^{a}	ND	15^{a}	4[3]
Nonanal	1104	430^{ab}	500^{ab}	180^{ab}	120^{b}	740^{a}	210^{ab}	320^{ab}	1[3]
(E)-2-Nonenal	1168	90^{a}	10^{bc}	60^{ab}	5^{c}	ND	ND	ND	0.15[3]
Decanal	1223	75^{ab}	240^{a}	10^{b}	40^{b}	30^{b}	60^{ab}	90^{ab}	5[3]
δ-Decalactone	1510	260^{a}	40^{a}	170^{a}	180^{a}	90^{a}	190^{a}	ND	7[3]

Compound	RI on DB-WAX[2]	Concentration (ppb)							Reported threshold (ppb)
		WPC 1	WPC 2	WPC 3	WPC 4	WPC 5	WPC 6	WPC 7	
Acetic acid	1441	850^{a}	2300^{a}	1300^{a}	2500^{a}	3000^{a}	3000^{a}	1500^{a}	22,000[3]
Butanoic acid	1538	1600^{b}	2800^{ab}	3200^{ab}	3600^{ab}	6600^{a}	2800^{b}	5200^{ab}	1000[3]
Pentanoic acid	1661	20^{c}	50^{c}	100^{c}	320^{c}	510^{bc}	960^{b}	3300^{a}	2100[3]
Hexanoic acid	1797	2000^{c}	2600^{bc}	2800^{bc}	8300^{a}	5700^{abc}	5500^{abc}	6300^{ab}	3000[3]
Heptanoic acid	1946	280^{b}	280^{b}	270^{b}	1100^{b}	870^{b}	3300^{a}	230^{b}	10,400[4]
Octanoic acid	2051	8500^{bc}	11,000^{b}	19,000^{a}	19,000^{a}	4900^{c}	4300^{c}	19,000^{a}	3000[3]
4-Methyl octanoic acid	2131	50^{b}	110^{b}	110^{b}	270^{ab}	220^{ab}	460^{a}	230^{ab}	600[5]
Nonanoic acid	2159	6800de	15,000^{cd}	5400de	2700^{e}	27,000^{b}	26,000^{bc}	43,000^{a}	8800[4]
Decanoic acid	2303	48,000^{b}	42,000^{bc}	51,000^{b}	108,000^{a}	4300^{c}	4500^{c}	3000^{c}	10,000[3]

$^{a-e}$Means in a row followed by different letters are different ($P < 0.05$).

[1]Retention indices (RI) calculated from mass spectrometry results on a DB-5 column.

[2]RI calculated from flame-ionization detector results on a DB-Wax column.

[3]Thresholds reported orthonasally in water (Rychlik et al., 1998).

[4]Thresholds reported in a potassium-hydrogen-phthalate buffer at pH 4.8 (Attaie and Richter, 1996).

[5]Thresholds reported in water at pH 4 to 5 (Brennard et al., 1989).

the characteristic soapy flavor, and so other compounds likely contribute to this flavor. Octanoic and decanoic acids were identified in WPI. These acids have been previously identified in whey (Tomaino et al., 2001). Octanoic and decanoic acids also have waxy/soapy aromas and were found at significantly higher concentrations ($P < 0.05$) in WPI samples 1 and 2, both of which had the soapy flavor. These data suggest that increased concentrations of octanoic and decanoic acids in WPI also contribute to waxy/soapy flavors.

There were many thermally generated compounds such as pyrrolines, pyrazines, and furanones. Again, this is expected because WPC 80 and WPI undergo spray drying in which residual lactose can react with protein to form Maillard reaction products or undergo caramelization reactions (Friedman, 1996; Mahajan et al., 2004). 2-Acetyl-1-pyrroline (tentative ID) had high $\log_3$ FD values in most samples. This compound has a very low odor threshold and contributes to cooked flavors in fresh fluid whey and fresh low-heat skim milk powders (Karagul-Yuceer et al., 2001, 2002, 2003a, 2004). 3-Hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon) also had high $\log_3$ FD values in all WPC 80. This compound was previously identified as a key aroma component of sweet whey powder (Mahajan et al., 2004). 2,5-Dimethyl-4-hydroxy-3-(2H)-furanone (furaneol) was found in some WPC 80 and WPI. This compound has also been previously identified in sweet whey powder (Mahajan et al., 2004) and is a sugar degradation product with a sweet, candy-like aroma. In general, these thermally generated compounds contribute to typical dairy flavors, such as cooked/milky and sweet aromatic. It is important to note their presence in WPC 80 and WPI, but our interest lies in understanding off-flavor formation, and these compounds are likely not sources of off-flavors in fresh dried whey products.

There were 4 sulfur compounds identified in the whey proteins. Methional, dimethyl sulfide, and dimethyl trisulfide have been previously identified in liquid and dried whey (Karagul-Yuceer et al., 2003a; Mahajan et al., 2004). Methional was one of the first compounds identified in dairy products and is a Strecker degradation product formed from the degradation of methionine. Dimethyl disulfide and dimethyl trisulfide have onion and cabbage odors, respectively. These compounds are formed from the degradation of methionine into dimethyl sulfide and methanethiol and then into dimethyl disulfide and dimethyl trisulfide (Bendall, 2001). Although the sulfur compound 2-methyl furanthiol (brothy/burnt) has not been previously found in whey, it has been previously identified in Cheddar cheese (Carunchia Whetstine et al., 2005) and is formed

Table 10. Relative abundance of selected compounds in whey protein isolate (WPI) samples (ND = not detected).

Compound	RI on DB-5MS[1]	Concentration (ppb)								Reported threshold (ppb)
		WPI 1	WPI 2	WPI 3	WPI 4	WPI 5	WPI 6	WPI 7	WPI 8	
Dimethyl disulfide	742	ND	ND	110^{ab}	147^{a}	110^{ab}	35^{ab}	ND	ND	0.16[3]
Hexanal	803	55^{a}	100^{a}	170^{a}	170^{a}	110^{a}	10^{a}	ND	ND	10.4[3]
Heptanal	913	65^{a}	ND	5^{a}	77^{a}	20^{a}	ND	ND	ND	3[3]
Octanal	1005	190^{a}	20^{ab}	5^{b}	20^{ab}	40^{ab}	ND	90^{ab}	80^{ab}	8[3]
Nonanal	1098	850^{a}	490^{a}	160^{a}	580^{a}	440^{a}	240^{a}	260^{a}	1100^{a}	1[3]
Decanal	1223	120^{a}	130^{a}	30^{a}	90^{a}	80^{a}	30^{a}	50^{a}	130^{a}	5[3]
Phenylethyl acetate	1245	ND	ND	ND	ND	35^{a}	ND	ND	ND	19[3]
(Z)-2-Decenal	1250	40^{a}	ND	ND	ND	40^{a}	ND	ND	20^{a}	0.4[3]
2-Undecanone	1290	20^{b}	ND	20^{b}	53^{a}	ND	ND	ND	18^{b}	3400[4]
2-Undecenal	1350	ND	ND	ND	ND	80^{a}	ND	ND	ND	Not reported
Dodecanal	1402	420^{b}	1287^{a}	ND	ND	ND	ND	ND	58^{b}	2[3]
δ-Decalactone	1510	ND	ND	ND	ND	ND	ND	ND	150^{a}	7[3]

Compound	RI on DB-WAX[2]	Concentration (ppb)								Reported threshold (ppb)
		WPC 1	WPC 2	WPC 3	WPC 4	WPC 5	WPC 6	WPC 7	WPC 8	
Acetic acid	1441	10,000^{a}	7900^{ab}	3400^{abc}	1700^{bc}	1300^{bc}	1400^{bc}	2300^{bc}	750^{c}	22,000[3]
Butanoic acid	1661	920^{a}	17,000^{a}	6200^{a}	13,000^{a}	6000^{a}	6800^{a}	2000^{a}	740^{a}	2100[3]
Pentanoic acid	1797	430^{b}	150^{b}	1800^{a}	810^{b}	540^{b}	600^{b}	140^{b}	380^{b}	3000[3]
Hexanoic acid	1946	23,000^{a}	11,300^{b}	1700^{d}	2600^{d}	4900^{cd}	3200^{cd}	9000^{bc}	6200^{bcd}	10,400[5]
Heptanoic acid	2039	1700^{a}	1600^{ab}	460^{c}	640^{bc}	380^{c}	1100^{abc}	330^{c}	850^{abc}	464[3]
Octanoic acid	2051	39,000^{a}	23,000^{b}	2700^{c}	4700^{c}	890^{c}	2500^{c}	2800^{c}	1600^{c}	3000[3]
4-Methyl octanoic acid	2131	1700^{a}	420^{a}	440^{a}	1100^{a}	330^{a}	170^{a}	320^{a}	1400^{a}	600[6]
Nonanoic acid	2159	26,000^{cd}	148,000^{a}	41,000^{bc}	63,000^{b}	27,000^{cd}	29,000^{bcd}	850^{d}	3800^{d}	8800[5]
Decanoic acid	2303	157,000^{a}	124,000^{ab}	25,000^{cd}	27,000^{cd}	44,000^{cd}	78,000^{bc}	11,000^{d}	9500^{d}	10,000[3]

$^{a-d}$Means in a row followed by different letters are different ($P < 0.05$).

[1]Retention indices (RI) calculated from mass spectrometry results on a DB-5 column.

[2]RI calculated from flame-ionization detector results on a DB-Wax column.

[3]Thresholds reported orthonasally in water (Rychlik et al., 1998).

[4]Threshold reported orthonasally in oil (Rychlik et al., 1998).

[5]Thresholds reported in a potassium-hydrogen-phthalate buffer at pH 4.8 (Attaie and Richter, 1996).

[6]Thresholds reported in water at pH 4 to 5 (Brennard et al., 1989).

from the degradation of sulfur-containing amino acids (Lee et al., 1996b). These sulfur compounds are sources of brothy flavors in Cheddar cheese (Singh et al., 2003) and probably contribute to these flavors in WPC 80 and WPI.

2-Phenethanol and phenyl ethyl acetate have not been previously identified in whey, but are found in aged Cheddar cheese and are formed from the Strecker degradation of aromatic amino acids, especially phenylalanine (Singh et al., 2003; Carunchia Whetstine et al., 2005). Another degradation product of phenylalanine, phenylacetaldehyde, has been previously documented in sweet whey powder (Mahajan et al., 2004). 2-Methoxyphenol (guaiacol) has a smoky aroma and was found in all WPC 80. This compound is found in aged cheese (Suriyaphan et al., 2001; Singh et al., 2003) and can be formed from the degradation of aromatic amino acids. 2-Isobutyl-3-methoxypyrazine, which has an earthy aroma, was identified in some WPC 80 samples. This compound was previously identified in liquid whey (Karagul-Yuceer et al., 2003a), and is responsible for the characteristic earthy aroma in British farmhouse Cheddar cheese (Suriyaphan et al., 2001). The degradation of amino acids can cause distinctive flavors in aged products, such as Cheddar cheese (Carunchia Whetstine et al., 2005), but the liberation of free amino acids is a slow process (Wallace and Fox, 1997). This is most likely why this mechanism of flavor formation is not as prevalent as lipid oxidation reactions in nonaged products such as whey, and why lipid oxidation products are the main sources of flavor (Tables 7 and 8; Swaisgood, 1996).

o-Aminoacetophenone has not been previously identified in WPC 80 or WPI, but was found in these samples. This compound has been documented in other dried dairy ingredients including skim milk powder and rennet casein (Karagul-Yuceer et al., 2002, 2003b). *o*-Aminoacetophenone has been reported to cause stale flavor in milk powders and is present at higher concentrations in stored skim milk powder compared with

fresh skim milk powder (Karagul-Yuceer et al., 2002). This compound is not likely a key flavor compound in fresh WPC 80 and WPI, but may be important in stored products. 2,3-Butanedione (diacetyl) has a buttery aroma and has been documented in many fermented dairy products including liquid whey and sweet whey powder (Karagul-Yuceer et al., 2003a; Mahajan et al., 2004). It was found in WPC 80 at low levels. This compound is produced during lactic fermentation by the oxidative decarboxylation of α-acetolactic acid (Cronin and Rispin, 1996).

There are several compounds in WPC 80 and WPI that are formed from triglycerides during the pasteurization of milk (Badings and Neeter, 1980; El Soda et al., 1995). These include δ-lactones and γ-lactones. Fresh raw milk does not contain lactones, but after pasteurization, lactones are formed (Dimick et al., 1969). γ-Nonalactone has not been previously found in whey but has been identified in skim powders (Karagul-Yuceer et al., 2002). Lactones generally display coconut or peach aromas (Singh et al., 2003). Based on aroma, there are several unknown compounds in the whey proteins that are likely lactones (Tables 6 and 7). The mechanism of lactone formation is nonoxidative and the precursors of these compounds are δ-hydroxy fatty acids esterified in milk fat (Dimick et al., 1969). Due to the lower amount of lipid found in WPI, there are fewer lactone compounds in these samples compared with WPC 80. 3-Methoxy-4-hydroxybenzaldehyde (vanillin) was also identified in WPC 80, but not in WPI. Vanillin originates (in the cow rumen) from plant lignin. This compound is formed during pasteurization (Cobb et al., 1963). Vanillin has not been previously identified in WPC 80 or WPI, but has been identified in skim milk powder (Karagul-Yuceer et al., 2001, 2002).

Tables 9 and 10 show the relative concentration of selected compounds. These compounds were selected for quantification based on their aroma potency as well as mass selective detection limits. Though other compounds may indeed be important to the flavor of WPC 80 and WPI, they were not present at high enough concentrations to be quantified instrumentally. There were no consistent differences in the relative abundances of the neutral/basic compounds between the samples, but WPI samples 1 and 2 had higher concentrations of fatty acids C8-C10.

There were more aroma-active compounds in WPC 80 than in WPI. However, there were not correspondingly more flavors or higher flavor intensities observed in the WPC 80 compared with the WPI. Morr and Ha (1991) reported that commercial WPI exhibited considerable "better" flavor quality than WPC 80. Their statement was based on qualitative observations and because WPC 80 contain more residual lactose, lipid, phospholipid, lipoprotein, copper, and other prooxidants that increase the likelihood of lipid oxidation occurring (Morr and Ha, 1991) compared with WPI. Indeed, WPC 80 contained higher mineral (ash) content (dry weight basis) than WPI, which may correspond to production or detection of more aroma-active compounds. Additionally, proteins can bind or trap volatile compounds (Quach et al., 1999) and protein, and thus volatile binding capacity is higher in WPI, which might explain the presence of more aroma-active compounds in WPC 80 than in WPI. However, in contrast to previous studies, close examination of the sensory profiles does not reveal a clear "better" flavor quality between WPC 80 and WPI. Dried dairy proteins, including WPC 80 and WPI, should ideally exhibit delicate bland flavors reminiscent of fresh fluid whey or milk. Cardboard/wet paper, pasta water, soapy, and cucumber flavors as well as bitter taste and astringency are not desirable sensory attributes in dairy products. Further work should address the formation of these undesirable nondairy flavors during processing as well as addressing flavor carry-through of these ingredients into product applications.

CONCLUSIONS

Before this study, there was no quantitative research examining the flavor of multiple WPC 80 and WPI from different manufacturers. This study sheds light on the compounds that contribute to WPC 80 and WPI flavor. Flavor variability (sensory and instrumental) was observed between and within WPC 80 and WPI. Mild dairy flavors (cooked/milky, sweet aromatic) as well as nondairy flavors (cardboard, pasta water, animal/wet dog) were documented in the rehydrated proteins. Lipid oxidation products in conjunction with heat-generated compounds were predominant sources of flavors. This research demonstrates the range and variability of aroma-active compounds and flavors in WPC 80 and WPI. Further research needs to be conducted to more fully aid the industry in providing consistent, bland WPC 80 and WPI ingredients.

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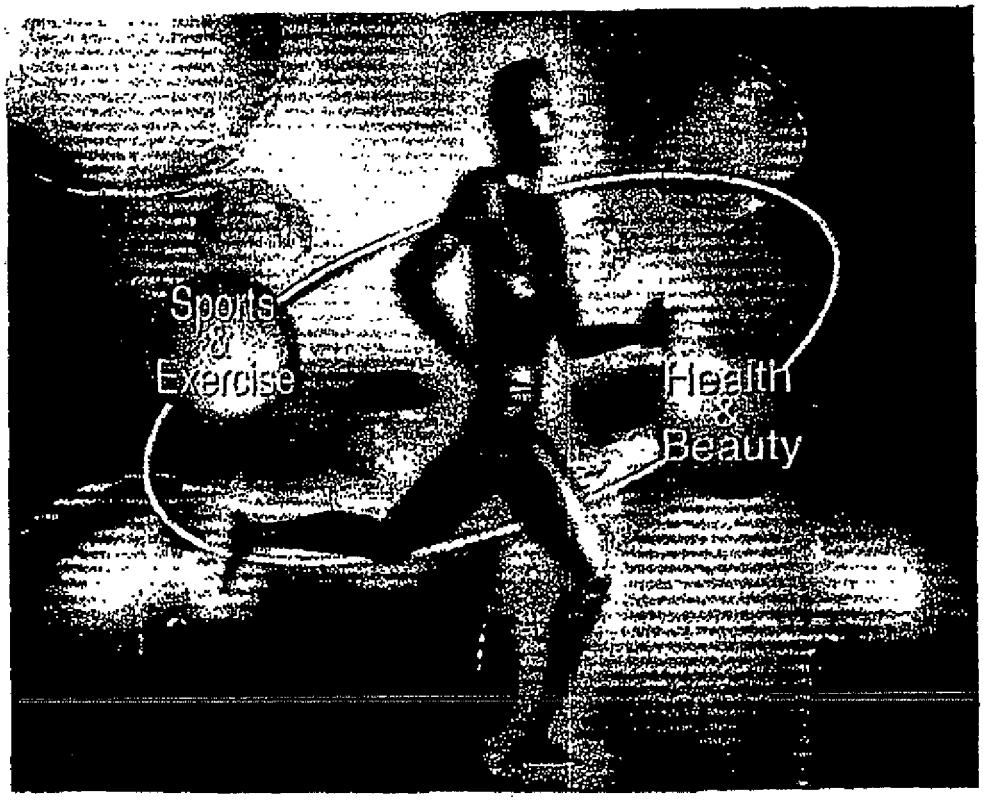
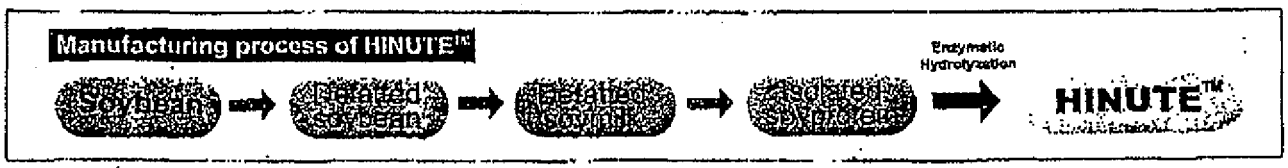


What is "HINUTE™"?

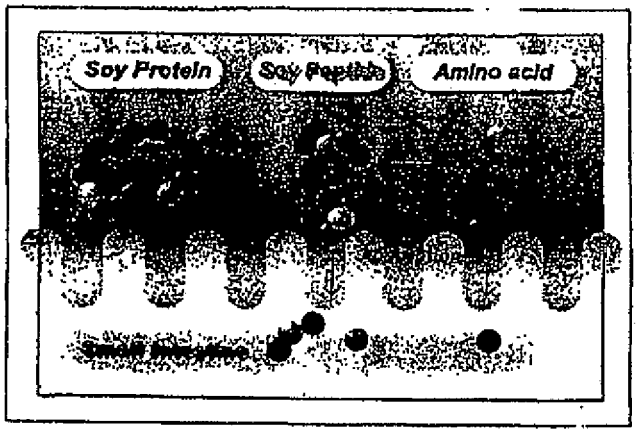
HINUTE is the trade name for our product of the partially hydrolyzed soy protein with our state-of-the-art enzymatic technology. It is rich in low molecular peptides* composed of well-balanced amino acids, but includes less free amino acids and less bitter taste.

It is so called a mixture of SOY PEPTIDES. HINUTE is absorbed easily and has a high nutritional value, so it can be applied in entrails nutrients, sports supplements, health foods and soft drinks etc.

*Peptide: Peptide is the family of molecules formed by the linking of various amino acids.



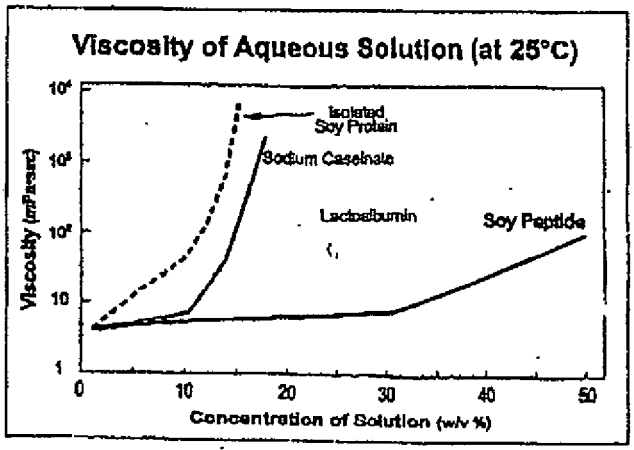
Characteristics of Soy Peptide



The soybean peptide is generated in the digestion process of soy protein.

It is known that the di/tri-peptides generated by the digestion is directly absorbed from small intestine in the form of peptide, and is thought its absorption is quicker than that of amino acids.

HINUTE contains many of soy oligo-peptides, and therefore, intake of HINUTE can swiftly provide well-balanced amino acids of soy protein to the human body.

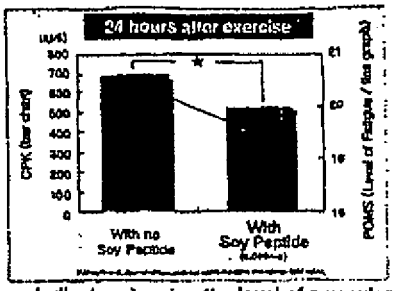


HINUTE is highly soluble/dispersible into water, and shows very low viscosity.

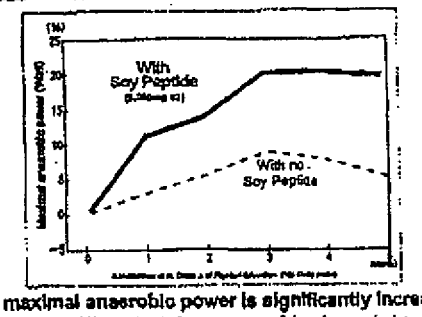
Foods and beverages, to which HINUTE is applied, will show little "filling" feeling of protein foods in general, and will bring excellent digestion/absorption.

HINUTE helps to recover the condition to exploit your abilities in daily life

Effect on exercise performance, and remaining musclar injury after exercise.

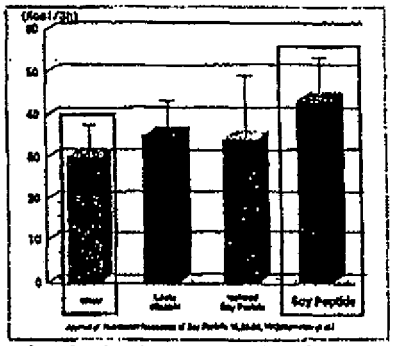


CPK is an indicator showing the level of muscular injury. When CPK value is small, the injury of muscle is small as there is no need to restore the muscle. (★:P<0.05)

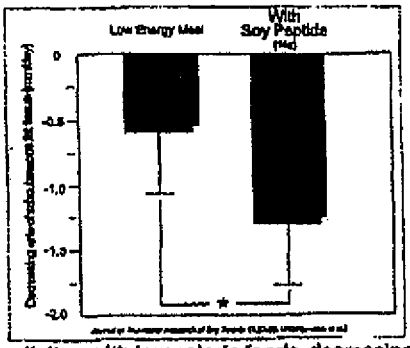


The maximal anaerobic power is significantly increased, but no significant differences of body weight were observed.

Effects of Soy Peptide consumption on diet induced thermogenesis.

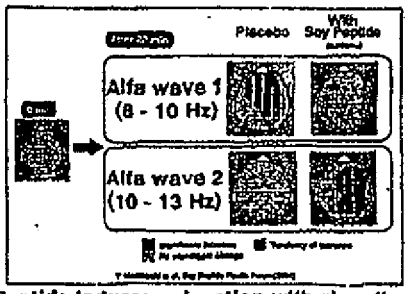


When taking the Soy Peptide, the basal fat metabolism is promoted and further, thermo genesis is induced at the surface of the body

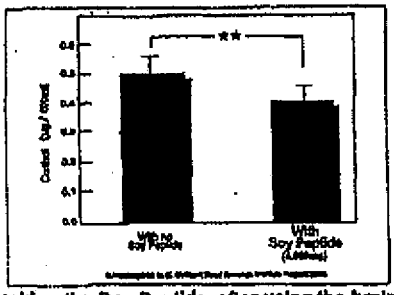


When dieting with low calorie foods, decreasing rate of subcutaneous fat tissue is accelerated by the intake of Soy peptide. (★:P<0.05)

Effect on brain activity.



Soy Peptide induces relaxation with clear thoughts maintaining the brain calm without sleepiness.



When taking the Soy Peptide, after using the brain, e.g. for calculation, the stress hormone tends to decrease. (★★:P<0.01)

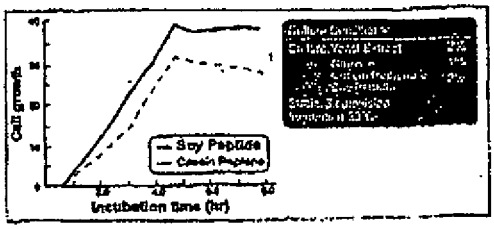
Fermentation Aid

Soy Peptide promotes the growth of various microorganisms as follows.

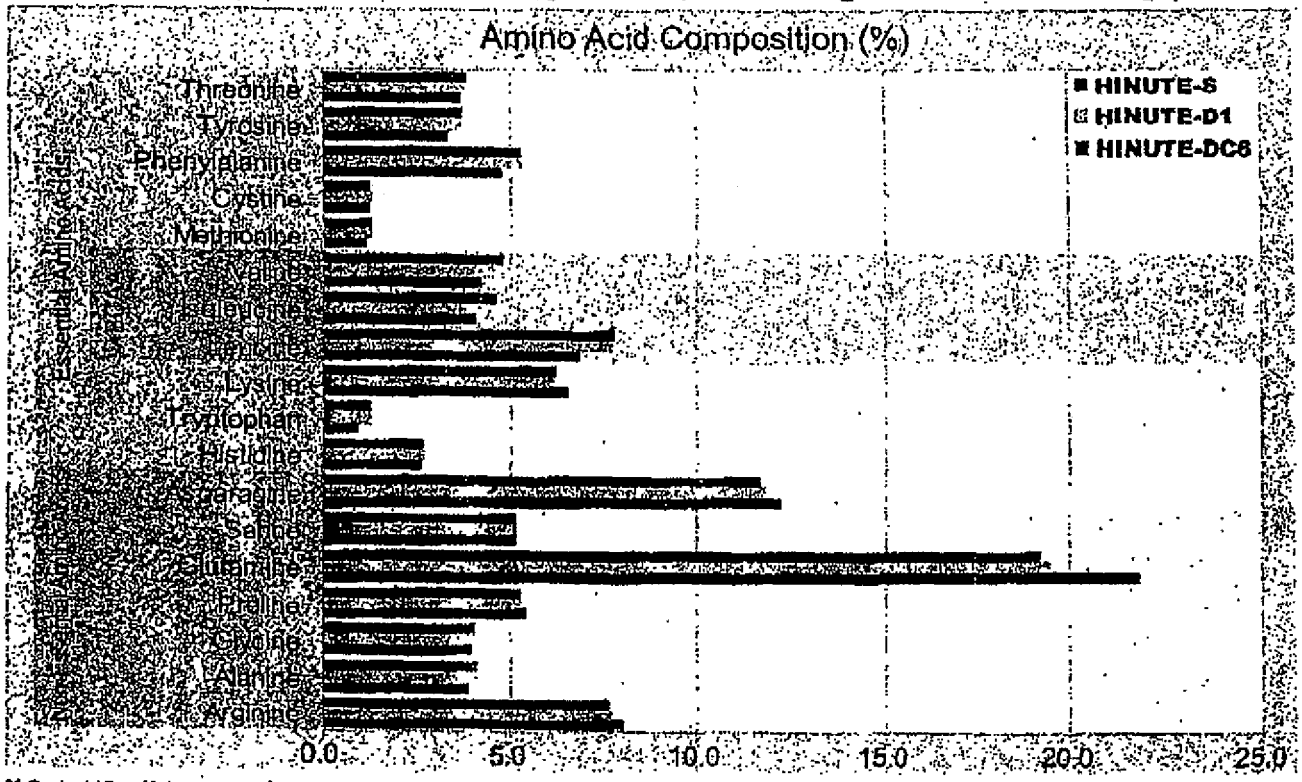
Effects for the growth of various microorganisms.

[illegible]	Growth promotion Strengthening stability
[illegible]	Growth promotion Raising CDx generation
[illegible]	Growth promotion Raising enzyme production ability
[illegible]	Growth promotion Raising enzyme production ability

Stimulating effect of Soy Peptide on the growth of *S.cerevisiae*.



Type	S	D1	DC6
Crude Protein (%)	≧80	≧80	≧75
Moisture (%)	≦7		
pH (10% Solution)	approx. 6.5		approx. 4.5 (Citric acid)
Appearance of Solution	Cloudy	Cloudy	Clear
Applications	Sports Nutrition Health Food Fermentation Aid	Sports Nutrition Health Food	Ready-to-drink beverage Powdered beverage
Shelf Life	360 Days		
Packaging	10kgs x 1, multi-layer paper bag, innerbarriercoated ONY		
Molecular Weight Distribution (%) ~ 500	71	54	75
500 ~ 12,000	12	17	9
12,000 ~	9	15	2
Mw	4,500	7,900	1,700
Free Amino Acid (%)	< 12	< 2	< 2



*1:Content / Dry *2:Asparagine+Aspartic Acid *3:Glutamine+Glutamic Acid

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Business & Products

Soy peptides

Produced from soy protein, soy peptides are rapidly absorbed into the body, helping to reduce muscle fatigue and boost physical strength. By stimulating the body to burn fat, they also help prevent obesity, making them ideal for products such as sports drinks and health foods.

Soy peptides

HI-NEUTE Series A soy protein oligopeptide rich in essential amino acids. It has nutritional value and excellent absorbency, enabling application in entral nutrient, sports supplements and health foods. Its non-precipitation quality under acidic conditions is ideal for an application in soft drinks.